

UTILITY PATENT APPLICATION
of
Ewa Herbst
for

TREATMENT OF LIVING TISSUES USING ELECTROMAGNETIC FIELDS

RELATED APPLICATIONS

This application is based on provisional application number 60/238414, filed 6 October 2000, the disclosure of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention.

This invention relates to apparatus and method for diagnosing and/or treating tissues using electromagnetic fields, and for determining therapeutically useful electromagnetic fields, and harmful electromagnetic fields.

2. The State of the Art.

Electromagnetic fields are used clinically for bone and wound healing and have been used experimentally to enhance nerve repair and/or regeneration. Most investigators have varied a very limited number of electric parameters, usually to show that the biological effect does exist, but without an attempt to optimize this effect. In contrast, the present invention undertakes a systematic approach to evaluate different signals and their components in a complex neuron explant model as a first step towards verification of the in vivo efficacy of such defined EM fields.

There are approximately 500,000 cases of nerve injury of upper extremity per year. Recovery from pain and restoration of function is slow and there is need

for more research in this area to speed up the whole process (Sisken and Walker, 1995).

The EMF signals which have been tested most extensively both *in vitro* and *in vivo* are 15 Hz pulse train as well as 72 Hz repetitive single pulse (EBI, Inc. Parsippany, NJ) and 2 Hz repetitive single pulse (Bietic Research, Inc. Lyndhurst, NJ) measured as a voltage induced in a small pick-up coil. A 2 Hz/3 Gauss field signal was tested *in vivo*, resulting in an increased axon elongation (Sisken et al, 1989) in the crushed rat sciatic nerve; the regeneration rate was enhanced by 22%, which is comparable to that reported in the literature with conditioning lesions, growth factors and hormones (Sisken et al, 1993).

In vitro results using 2 Hz/0.5 Gauss EMF on the cultured dorsal root ganglia (DRG) explants showed significantly increased neurite outgrowth (Sisken et al, 1990). The DRG explants in culture is a well-established *in vitro* model to study effects of different factors on nerve regeneration (Greenebaum et al, 1994). The chick DRG has been used as a model system testing for growth factor effects and mechanisms for the past 40 years (Levi-Montalcini, 1968). The dorsal root ganglion is the sensory part of the sensory-motor axis; sensory neurons are in the ganglia whereas motor neurons are in the ventral portion of the spinal cord

The equipment used most often for *in vitro* and *in vivo* studies of low frequency electromagnetic field effects on nerve regeneration was developed originally for bone healing (Electro-Biology, Inc., Parsippany, NJ) and delivers either a repetitive 72 Hz single pulse or a pulse train with a repetition rate of 15 Hz. Nerve regeneration studies using this equipment were performed by: Ito and Bassett; Orgel et al; and Subramanian et al. The RF signal Diapulse system has also been used for nerve regeneration experiments (Wilson; Raji and Bowden). Some investigators (Blackman; Rusovan et al; Subramanian et al) used various sinusoidal EMFs. A repetitive single pulse system by Bietic Research, Inc. (Lyndhurst, NJ) has also been used in wound healing and nerve regeneration

experiments (Sisken et al; Orgel et al). For review of the above studies, see Sisken (1991).

SUMMARY OF THE INVENTION

In light of the foregoing, one object of this invention is to provide a method for determining therapeutic as well as harmful values of B and/or dB/dt specific to a given cell, tissue type, tissue system (plants), or microorganism (such as, for example, bacteria and yeast). As used herein and in the claims, the term "tissue" refers to all of these cells, tissue types, tissue systems, and microorganisms.

Yet another object of this invention is to provide an apparatus for delivering a therapeutic B and/or dB/dt to a given cell, tissue type, tissue system (plants), or microorganism (such as, for example, bacteria and yeast). Yet still another object of this invention is to provide an apparatus for delivering a B and/or dB/dt to a given cell, tissue type, tissue system (plants), or microorganism (such as, for example, bacteria and yeast) to affect a change in that tissue's state or function, whether beneficial or detrimental.

Still a further object of the present invention is to provide a method for avoiding a harmful value of B and/or dB/dt.

In one embodiment, this invention provides a method for treating living tissues with electromagnetic fields by subjecting the tissues to be treated with an electromagnetic field that varies as a function of time, and without the need for a corresponding static magnetic field.

In another embodiment, this invention provides a method for avoiding harmful values of dB/dt by recording dB/dt values in a given environment, reproducing those values in cells, tissues, tissue system (plants), or microorganism (such as, for example, bacteria and yeast) *in vitro* and/or *in vivo* to determine the effects, and if such effects are detrimental, then operating and/or modifying and/or

designing the equipment and/or circuitry generating the harmful B and/or dB/dt values in the given environment in a manner that avoids the harmful values.

BRIEF DESCRIPTION OF THE FIGURE

Fig. 1 depicts three different electromagnetic field signals tested in this invention.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS

All of the prior art systems described in the Background section above have a voltage output stage powering the coils, which makes it difficult to set up experiments in which B (magnetic flux density) and dB/dt (time rate change of B), as well as the shape of the electric field E, which depends on dB/dt, can be well-defined *a priori*. This is due to the fact that the B field induced inside the coils is a function of the current flowing through the coils and has no linear relationship with the output voltage. Accordingly, in this invention it is preferred to control the current flowing through the coils, thereby avoiding the problems when attempts are made to provide a specified B field by controlling the voltage.

In the experiments described herein, the magnetic flux density B is controlled directly by controlling current from an electromagnetic field generating system. The system also allows for control of dB/dt variations and, consequently, for changing the parameters of the electric field in the treated area in a well controlled manner. Once the geometry and the number of turns of the coils have been specified, B is strictly proportional to the current I with a proportionality constant specific for the coil system used. This allows one to *a priori* specify B field in the experiments, by specifying current I. By changing one of the parameters (maximum magnetic flux density or its time characteristics) while keeping the other constant, dB/dt can be changed in a controlled way as well.

The impact of various dB/dt rates on both positive and negative biological responses can be of special importance, as the time rate change of the magnetic flux density may have effect on the cell membranes. The biological responses to electric and/or magnetic fields, and specifically to dB/dt, may influence healing processes in the body, as well as cell damage, depending on the levels and timing characteristics of the signals involved. To our knowledge, no systematic study has been performed to evaluate dB/dt effects on biological processes, specifically on tissue healing and/or regeneration.

As electric and magnetic fields cannot be separated from each other and investigated independently, it is difficult to find a suitable model for studying their respective influence on biological tissue. One such approach is to investigate effects of electric field *in vitro* at the different radial distances from the center of a culture dish (Misakian et al, 1990, Bassen et al 1992) in a presence of identical magnetic flux density throughout the whole surface area of the dish. In the center of the dish the induced electric field is equal to zero and it reaches its maximum at the edge of the dish. The resulting current density \mathbf{j} is equal to the conductivity of the media σ times the electric field \mathbf{E} : $\mathbf{j} = \sigma \mathbf{E}$. In earlier experiments by Siskin et al (1984), the resulting current density for 15 Hz pulse train signal (EBI, Parsippany, NJ) was $5 \mu\text{A}/\text{cm}^2$ at the distance of 2 cm from the center of the dish. By placing DRGs at various specified distances from the center of the dish, the biological effects of electric fields of various amplitudes on DRGs can be studied in the presence of the same magnetic flux density. This approach allows one to differentiate biological effects due to the electric field only, as it increases proportionally to the distance from the center of the dish, while all other EMF parameters and culture conditions remain equal.

For a true Helmholtz coil, the magnetic flux density B on the coil axis at half the distance between the coils is: $B = \mu_0 NI/1.4a$; wherein μ_0 is permeability for a vacuum, N is the number of turns of each coil, I is the current flowing through each

coil, and a is the coil radius equal to the distance between the coils. Once the geometry and the number of turns of the coils have been specified, B is strictly proportional to the current I with a proportionality constant specific for the coil system used. This allows one to *a priori* specify B field in the experiments, by specifying current I . By changing one of the parameters (maximum magnetic flux density or its time characteristics, for example) while keeping the other constant, dB/dt can be changed in a controlled way as well. In addition to Helmholtz coils, various other coil geometries, such as a saddle, a helix, or the like as are presently used can be used for this invention, and optionally multiple coils and/or coil geometries can be used in this invention.

To be able to answer questions about signal specificity, several electrical parameters have to be investigated, one at a time, in a consistent and logical order. These parameters include, without limitation: type (shape) of the signal; signal amplitude, signal frequency; for pulses, a single pulse or pulse train; time characteristics of the pulse, time rate of change of magnetic flux density, etc. Both amplitude of the field induced in the tissue and time characteristics of the signal are of importance, while discussing kinetics of biological responses at the cell membrane.

The present invention is not limited to the use of a preferred current output system (current output amplifier), as long as the above signal considerations are taken into account.

Signal Generator And Power Amplifier Of The Invention

The microprocessor-controlled signal generator for three specific signals (Fig. 1), which were used in the experiments described below, is designed around an AM186ES 40-MHZ microcontroller. A suitable signal generator is disclosed in US 6,029,090, the disclosure of which is incorporated herein by reference. The chosen signal is fed into a current output power amplifier, such as one with peak

output currents in the range of about ± 50 A, a maximum output voltage of up to a few thousand volts, and with a bandwidth of up to a few megahertz. The amplifier can have a built-in inductance load over-voltage protection, as well as a current limit for the current amplifier and an open load protection. It can be used with resistive and capacitive loads, in addition to inductive loads such as the coils of this invention.

The resulting stimulator system preferably incorporates safety features that protect the equipment from damage if the coil is not attached when the power is turned on. The current output power amplifier allows the opportunity to define *a priori*, and to accurately control, the magnetic flux density B , which is directly proportional to the current, as well as the time derivative of the magnetic flux density dB/dt and the resulting electric field E . A primary consideration with the development of the stimulator's universal power amplifier for generating signals useful in this invention is that it must be both linear and fast, in order to take care of all the different signals. Additionally, it must be able to deliver a wide range of currents, depending on the type of the signal and its time characteristics and amplitude, as well as the size of the coils. Depending on the current and the inductance of the coil/s, the voltage available at the output of the stimulator will vary significantly.

Coil Of The Experimental Setup

The following description specifies a Helmholtz coil configuration, although other coil configurations can be used instead, if more appropriate for a specific application. For the experiments described herein, the coil design had to take into account both the bandwidth of the signal and a requirement for not increasing the temperature at the culture site. The number of turns of the coil wire depends on the optimal solution for the whole stimulator system with respect to minimum and maximum current amplitudes permitted in the coils as well as the bandwidth of the

signal. A desirable maximum temperature increase at the treatment site of less than 0.5°C, due to the heat development in the coils, provided a limitation on the maximum current allowed; in fact, the final design of the coil used in these experiments exceeded the inventor's own specifications, and resulted in a temperature increase in the culture media in the middle of the coil system of less than 0.1°C for less than 0.75A of current.

Two sets of rectangular Helmholtz-type coils 30 cm x 30 cm were designed and built. In order to use the coils in the future under various experimental conditions, each half of the Helmholtz coil consisted of two 33-turn coils. Such a design allows for four different system configurations: in series to double the number of turns and limit current and power dissipation in the coils; in parallel to decrease the inductance and increase the bandwidth of the Helmholtz coils; not connected at all and used as a 33-turn Helmholtz coil system; or not connected and powered by two identical current sources, with current in each 33-turn Helmholtz coil system flowing in the opposite direction to cancel the field inside the Helmholtz coil.

In these experiments, both halves of the Helmholtz coils were connected in series to assure the same current from the current-output stage of the power amplifier. Great care was taken to design a coil system that could be used for both higher and lower frequencies, thus taking into account a skin effect at higher frequencies and power dissipation in the coils at lower frequencies.

A Litz wire NELC150/36SPDN-1 (New England Electric Wire Corporation, Lisbon, New Hampshire) was used for coil manufacturing. This wire, which is recommended for frequencies 20-50 kHz, was sufficient for the initial experiments from a frequency point of view. Ideally, a coil bandwidth higher than the given power amplifier's bandwidth is desirable, which can be achieved with a higher frequency Litz wire.

A compromise was struck in terms of a desirable equivalent AWG (i.e., an equivalent wire diameter), due to the fact that a larger-diameter wire would not fit under the incubator's door for the present experimental setup. To compensate for the smaller diameter AWG used, as well as the resulting increase in energy dissipation and a potential heating of the wire, larger number of turns was required. Since the frequency was not critical for the initial experiments, the coils in each half of the Helmholtz configuration were connected in series, resulting in a 66-turn coil, and thus minimizing the current into the system. The total DC resistance of the Helmholtz coil, including the connecting wires to the stimulator, was measured at 2.4 Ohms.

Applications of the Method and Apparatus of the Invention

The experiments described below represent as a special case, peripheral nerve regeneration *in vitro*, but the invention is not limited to the peripheral nerve regeneration. Examples of other application areas are, without limitation: repair and/or regeneration of central nervous system; effects on autonomous nervous system; wound healing; other soft tissue healing and/or repair; tendon repair; cartilage repair; bone healing and/or repair; positive and negative effects on immune system function; disinfecting solid, liquid, and gas media; as well as other physiological, cellular, and biochemical effects. Yet other applications may include design of power stations and other work places, as well as machinery and instruments, to prevent harmful effects of high-level transients, due to switching of electric and/or magnetic fields. In addition, effects on plant development, growth, and function and/or on their pests and/or symbionts can be achieved, such as by burying coils in the soil (preferably powered by solar radiation, and optionally with batteries storing the solar power for release into the coils at the appropriate time).

Depending on the application, the electrical as well as timing and/or frequency parameters of both the magnetic flux density B and its time derivative

dB/dt will have different usable ranges. For example, to stimulate healing and/or regenerative processes in living tissues the magnetic flux density ranges are likely between the geomagnetic field up to about 10 mT, depending on the type of the tissue and whether the effects are sought in a cell culture, a small animal (such as a rat or cat), or a larger animal (such as a human or horse). The harmful effects of B and/or dB/dt may likely also start within this range, again depending on the type of the tissue and the size of the living organism and will continue for higher fields and/or dB/dt. The timing parameters alone may be of importance, as well as being a defining factor for the value of dB/dt and the resulting peak electric field E, which is a function of dB/dt. The frequency range for the beneficial effects may be from the low Hz range up to 100 kHz or higher, and may be up to the MHz range for some specific applications and combinations with specific field amplitudes. In most cases it will be under 10 kHz. This again may depend on the application area and the type of the organism treated. The frequency ranges for harmful effects will typically start within 100 kHz range and go up, again it may depend on the combinations of B and the timing characteristics of the signal employed.

EXPERIMENTS USING METHOD AND APPARATUS OF THE INVENTION

Tissue Cultures

The chick embryo nervous system was used to test for neurite-promoting activities of EMFs in culture. (Sisken et al., 1990; Greenebaum et al., 1996.) In these tests, sensory parent neurons of typical peripheral nerves (i.e., dorsal root ganglia (DRG) sensory neurons) were cultured. DRG dissected from 8-1/2- to 9-day chick embryos were explanted to 60-mm culture dishes coated with rat tail collagen. Explants, rather than single cells, were used to determine the effects on neurite growth since the presence of the highly important non-neuronal cells in close approximation to neurons was maintained.

Six dishes/groups with 12 DRG per dish were used in each experiment; one group served as a sham control and one as an EMF exposed group. Both groups were matched to have comparable concentrations of nerve growth factor (NGF) added. The NGF concentrations used were 0 ng/cc, 2 ng/cc, and 50 ng/cc (with two dishes per each concentration), to look at the effects of EMFs at low versus high NGF concentrations. The cultures were fed with neurobasal and N2 supplement (Gibco Co., NY). They were cultured for 48 hours at 37°C and 95% air, 5% CO₂, before fixation with phosphate-buffered formalin.

DRG Preparation

Under sterile conditions, 8½ to 9-day-old chick embryos were obtained and placed in phosphate-buffered saline (PBS) with pen/strep added. The embryos were then dissected under a dissecting microscope at low power. The DRG were then explanted from the embryos and placed in Falcon 60-mm-diameter culture dishes. A slight film of media was present on the bottom of the dishes to aid in the adhesion of the DRG to the bottom of the dishes.

The DRG were positioned in the culture dishes at two different radial distances from the center of the dish. Four DRG were placed at about 5 mm from the center and the other eight on the circle at 20 mm from the center. This approach gave a four-fold difference in the amplitude of the induced electric field, as well as in the current density.

A total of 12 dishes were used per experiment (6 controls, 6 treated). The dishes were placed in an incubator for 2 hr before the remaining media was added. This was done to further promote DRG adhesion to the bottom of the dishes. After this 2-hr period, NGF and media were added to the appropriate dishes. At this time, in order to keep the evaluators blinded, the code used in this experiment was written on top of both dish covers.

Experimental Set-Up

Each experiment was run for two consecutive days, with cultures exposed to EMFs for two hours/day, and was repeated seven to eight times, to assure enough data for statistical analysis.

5 All six EMF-treated culture dishes were placed on one shelf, centrally located in a 30 cm x 30 cm Helmholtz coil housed in the bottom chamber of a two-chamber incubator. The other six dishes (controls) were placed in the top incubator. The coil was placed parallel to the bottom of the culture dishes, resulting in an induced B field perpendicular to the surface of the dish. This
10 arrangement guaranteed identical magnetic fields in each dish, and was confirmed by measurement.

The coils were designed in such a way that a maximum temperature increase at the culture site, due to the heat development in the coils, would be less than 0.5°C. The temperature was monitored during the experiments with a non-metal, alcohol thermometer placed in front of the coil, and checked also in a spare
15 dish with culture media inside the coil (it was measured to be less than 0.1 °C for the current amplitude of 0.75A used in the experiments). The temperature inside the culture dishes was not monitored during the experiments due to the contamination risk.

20 Coils in the experimental system were connected to the EMF stimulator outside the chamber, consisting of the microprocessor-controlled signal generator and a current output power amplifier with peak output current in the range of $\pm 10A$, a maximum output voltage of up to $\pm 50 V$, and with a bandwidth of 50 kHz.

The evaluation was handled in a blinded fashion by an independent
25 statistician. Only the person who handled the exposure system and measured the fields daily knew which coil was active and what field was applied. This person did not evaluate the results.

Three different signals were tested sequentially, rather than in parallel, since access to three independent incubators was not possible; however, the evaluation of the results was done at the same time. All electrical parameters were measured during each experiment using a small search-coil attached to an oscilloscope to measure an induced electric field and a magnetometer to measure the ambient field. The magnetometer was also used to calibrate the system by comparing its reading to the calculated value of B, based on the value of current flowing through the coils. Once the calibration factor was defined, the measurement of current in the coils was sufficient to define the B field.

Electromagnetic Fields Tested

The efficacy of three electromagnetic fields (Fig. 1) on nerve regeneration *in vitro* was compared in a feasibility study. The peak magnetic flux density was constant for all three fields and equal to 0.3 mT.

The first field tested was a square pulse magnetic field ($B_{1\text{peak}} = 0.3 \text{ mT}$, pulse width = 20 ms, repetition rate = 2 Hz), imitating the electrical parameters of the Bietic Research, Inc. system that showed positive effects on nerve regeneration *in vivo* in the crushed sciatic nerve in rats (Sisken et al, 1989). The dB/dt for Bietic Research 2Hz/0.3mT field, based on the inventor's calculations using measured values of the voltage induced in the search coil, was in the range 0.32T/s to 0.54T/s, depending on the rise or fall time of the B field.

For these experiment with a square pulse magnetic field, both rise and fall times were set to approximately 1 ms, which is in the range of the original Bietic Research signal (0.9-2ms). This results in dB/dt=0.3T/s, $E=-0.03\text{V/m}$ at the 2 cm distance from the center of the dish, and $j=0.51 \mu\text{A/cm}^2$. This first experiment sought to define if the field amplitude, which affects the nerve regeneration *in vivo* in a rat sciatic nerve model, would also be effective *in vitro*. If

not, lower signal amplitudes were to be used, as earlier experiments showed that 0.05 mT has been effective *in vitro* (Sisken, 1990).

The additional two fields tested (Fig. 1) had their electrical parameters modified in such a way that additional preliminary data could be obtained to indicate appropriate experimental directions for the Phase II investigation. In both cases both the rise time and the positive dB/dt were identical to those in the first signal. In signal r the fall time was about 2.5 times shorter, resulting in a higher negative dB/dt. Signal t had symmetrical rise and fall times of 1 ms.

Evaluation Methods

Assessment of Neurite Outgrowth. DRG evaluation began by taking pictures using a 33-mm SLR camera (with no lenses), mounted directly to a microscope. Color-print film was used. The microscope objective was at 4x magnification, which meant that more than one picture had to be taken of larger DRG. Once the film was developed, the multiple pictures were assembled into a montage for each DRG. It should be noted that a total magnification factor of 46 was present when the measurements were made. This magnification factor was determined using a calibrated microscope slide by taking pictures under the same magnification and developing and printing the photographs under the same procedures.

From these pictures, DRG neurite length (i.e, the distance measured in a straight line from the main body of the DRG to the tip of each neurite) and the number of neurites per DRG were determined. Length measurements were made on each quadrant using a metric rule with 1-mm divisions. The main body of the DRG, containing the neuronal cell bodies, was easily distinguishable and outlined free-hand with a permanent marker. The mean neurite length for each DRG was also calculated.

Measurements were made using a jeweler's large magnifying glass, so that both picture and ruler were magnified simultaneously. The observer then

measured the distance from each neurite tip to the outline of the DRG main body, calling out the length measurements and recording them on a tape recorder. The neurite number was then simply a matter of recording the number of neurite length measurements for each DRG.

5 Tape-recorded measurements were then input into computer and analyzed using SAS (Statistical Analysis System). All of the measurements were made in a blinded fashion, by a technician who was not involved in culturing the DRG or in treating the DRG with EMFs.

10 Statistical Analysis. Mean neurite length and mean number of neurites was computed for each location (inner and outer DRG) in each dish. Then a series of linear mixed models (Verbeke and Molenberghs, 1997) was fitted to these data. A mixed model was used because there are two sources of variability in this experiment: the first is dish-to-dish variability, while the second is within-dish variability. A separate mixed model was used for each of the three signals. In
15 each model, the between-dish factors were: NGF concentration, which appeared at three levels (labeled 1 (0 ng/cc), 2 (2 ng/cc), and 3 (50 ng/cc)); treatment, which appeared at two levels (the active signal versus control labeled as t and c); and the interaction between concentration and treatment. The within-dish fixed-effects factor was location in the dish (inner versus outer DRG labeled as I and O) and all
20 two-factor and three-factor interactions between location and concentration and/or treatment.

 Also, an analysis was conducted to compare signals by pooling the data into a larger mixed model, which incorporated signal as another fixed factor in the between-dish effect (as well as interactions with all of the above stated fixed
25 effects). Finally, in all these mixed models, day of experiment (labeled as R for replicate) was treated as a blocking factor.

Statistical significance for all main effects and all interaction effects was set at 0.05. If a main effect or an interaction among these effects was statistically significant, then post hoc comparison of mean response was based on Fisher's least significant difference procedure; this was facilitated by using least squares means. All arithmetic was done using Procedure Means and Procedure Mixed in SAS.

Results

For neurite length and signal r there is a significant effect due to the interaction of treatment and concentration. Post hoc comparison of means shows that the mean response to the signal r is larger than the mean response for control at the 2nd concentration ($P = 0.0002$) but there is no difference between treatment and control at the remaining two concentrations. The following table shows the magnitude of the effects.

Table 1: mean neurite length (in parenthesis standard error) for signal r and comparison to its control

treatment	concentration		
	1	2	3
signal r	0.633 (0.035)	1.040 (0.032)	0.858 (0.033)
control	0.668 (0.032)	0.864 (0.032)	0.834 (0.032)
P value	0.47	0.0002	0.61

For neurite length and signal r there is also a difference between the mean response for inner versus outer DRG, where the mean response for inner 0.849 (± 0.019) is significantly larger than the mean response for outer 0.782 (± 0.019) with $P = 0.013$.

Discussion

A highly significant difference in the neurite length ($P=0.0002$) for one signal, with respect to its control (at an NGF concentration of 2ng/cc), but not for the other two signals, indicates that there may possibly be a signal-dependent effect on mean neurite length in the DRG model. Additionally, the fact that the mean neurite length for inner DRG was larger than for the outer DRG would indicate a possibility that the electric field induced in the outer ring was too high for an optimal biological response. This could possibly explain a lack of results with a pulsed B field, which gave a response in an earlier study. We used an amplitude of 0.3 mT taken from an *in vivo* experiment. In a previous *in vitro* experiment the amplitude was 6 times

lower. Also the time of the original experiment was longer, which can possibly have an impact on the outcome.

REFERENCES

The following documents in their entirety are expressly incorporated by
5 reference herein:

Bassen, H., Litovitz, T., Penafiel, M. and R. Meister. ELF in vitro exposure systems for inducing uniform electric and magnetic fields in cell culture media. Bioelectromagnetics 13:183-198, 1992.

Greenebaum, B, Sutton, Carl, M Subramanian Vadula, J H Battocletti, T Swiontek, J DeKeyser, and B F Sisken. Effects of pulsed magnetic fields on neurite outgrowth from chick embryo ganglia. Bioelectromagnetics 17: 293-302, 1996.

Herbst, E. Response of rat skin flaps to sinusoidal electromagnetic fields. Proceedings of the 9th Annual Conference of the IEEE Engineering in Medicine and Biology Society, vol. 9:75-76, 1987.

Herbst, E. Multifunctional Electric and/or Electromagnetic Stimulator. Proceedings of the Second World Congress for Electricity and Magnetism in Biology and Medicine, June 1997.

Levi-Montalcini, R and Angeletti, PU 1968. Nerve growth factor. Physiol. Rev. 48:534-569.

Misakian, M. and WT Kaune. Optimal experimental design for in vitro studies with ELF magnetic fields. *Bioelectromagnetics* 11:251-255, 1990.

Shah, A.J., C. Fischer, C.F. Knapp and B.F. Sisken. Determination of neurite outgrowth of dorsal root ganglia using image processing. *Proceedings of the 20th Annual Meeting of the Bioelectromagnetics Society*, June 1998.

Sisken, BF, B. McLeod and AA Pilla. PEMF, direct current and neuronal regeneration: effect of field geometry and current density. *J.Bioelectricity*, 3:81, 1984

Sisken, BF, M Kanje, G Lundborg and E Herbst. Stimulation of rat nerve regeneration with pulsed electromagnetic fields. *Brain Research* 485:309, 1989.

Sisken, BF, M Kanje, G Lundborg, and Kurtz, W. Pulsed electromagnetic fields stimulate nerve regeneration in vitro and in vitro. *Restor. Neurol. and Neurosc.*1:303-309,1990.

Sisken, BF. Electric and pulsed electromagnetic field effects on nerve tissue regeneration. In: *Electromagnetics in Medicine and Biology*. Eds CBrighton &SPollack,San Fran.Press, pp 259- 274, 1991.

Sisken, BF, J Walker and M Orgel. Prospects on Clinical Applications of Electrical Stimulation for Nerve Regeneration. *J Cellular Biochem.* 52: 404-409, 1992.

Sisken, BF and J Walker. Therapeutic aspects of electromagnetic fields for soft tissue healing. Chapter in *Electromagnetic Fields: Biological Interactions and Mechanisms* In: *The Amer. Chemical Society Series 250*, Editor Martin Blank, Amer. Chem. Soc., Washington DC 1995.

Subramanian M, C Sutton, B Greenebaum and BF Sisken. Interaction of pulsed electromagnetic fields and nerve growth factor on nerve regeneration in vitro. In: Electromagnetics in Medicine and Biology.(Eds.) C Brighton and S Pollack, San Francisco Press, 145-152, 1991.

5 Verbeke, G. and Molenberghs, G. Linear Models in Practice, Springer Verlag, 1997.

Walker, J, JM Evans, P Resig, S Guarnieri, P Meade and BF Sisken. Enhancement of functional recovery following crush lesion to the rat sciatic nerve by exposure to pulsed electromagnetic fields. *Exper. Neurol.* 125: 302-305, 1994a.

10 Walker JL, Evans JM, Meade P, Resig P, Sisken BF: "Gait-Stance Duration as a Measure of Injury and Recovery in the Rat Sciatic Nerve Model" *Journal of Neuroscience Methods* 52:47-52, 1994b.

15 Walker JL, Resig P, Guarnieri S, Sisken BF, Evans JM: "Improved Footprint Analysis Using Video Recording to Assess Functional Recovery Following Injury to the Rat Sciatic Nerve", *Restorative Neurology and Neuroscience* 6:189-193, 1994c.

Zienowicz, RJ, B Thomas, W Kurtz and M Orgel. A multivariate approach to the treatment of peripheral nerve transaction injury: the role of electromagnetic field therapy. *Plastic and Reconstr. Surgery*, 87: 122-129, 1991.